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A Applicant(s):	Karen Newell
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Examiner:	Amy M. DeCloux
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CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 21 day of August, 2003.

Helen C. Lockhart

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. MARTHA KAREN NEWELL

I, Martha Karen Newell, Ph.D., declare as follows:

1. I make this declaration in support of U.S. Serial No. 09/277,575 on which I am named as the sole inventor.

2. I am an Associate Professor of Biology at the University of Colorado in Colorado Springs. I have been performing research in the field of immunology generally and, more specifically, in the area of MHC class II mediated signaling and cellular apoptosis for many years.

3. I have performed many experiments in my laboratory which relate to my discovery of the relationship between the metabolic state of the cell and the immune system. In particular

much of my research has focused on the ability of molecules such as fatty acids to stimulate immune molecule expression in cells such as tumor cells.

4. My invention is based at least in part on the finding that fatty acids modulate cell surface immune molecule expression, and that fatty acids may be used to induce expression of immune molecules such as HLA-DR on the surface of a tumor cell.

5. In order to demonstrate that fatty acids may be used to induce expression of immune molecules such as HLA-DR on the surface of a tumor cell, I have conducted several experiments examining the impact of oleoyl S-CoA on MHC class II expression. The results of some of these experiments are presented in Figures 1 and 2.

6. Addition of exogenous oleoyl S-CoA increases expression of HLA-DR (MHC class II) in cells adapted a low lipid environment. Figure 1 is a graph depicting the effect of exogenous oleoyl S-CoA increases expression of cell surface HLA-DR on low lipid 60-7 cells. 60-7/LL cells were incubated for 15-18 hours in low lipid medium without (solid bar) and with (hatched bar) addition of 20 μM oleoyl S-CoA. Cell surface expression of HLA-DR was determined by immunocytometry using PE-conjugated anti-human HLA-DR antibodies. Results are expressed as the geometric mean fluorescence of stained samples/isotype controls.

7. Figure 2 is a graph depicting a time course of HLA-DR expression. 60-7/LL cells were incubated in low lipid medium with and without the addition of 20 μM oleoyl S-CoA. At specified times during the incubation, cells were removed and assayed for lysosomal acidity (LysoSensor), cell surface HLA-DR (immunostaining on intact cells), and total cellular HLA-DR (immunostaining on permeabilized cells) as described in Figure 1. Results are expressed as the geometric mean fluorescence of stained samples/no stain (LysoSensor) or isotype (HLA-DR staining) controls.

8. The results demonstrate that exogenous fatty acid stimulates expression of HLA-DR in cell lines adapted for growth in low lipid medium. Therefore, exposure of cells to fatty acids results in the induction of MHC class II on the cell surface.

8. I, Martha Karen Newell, Ph.D, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under §1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

August 20__, 2003

Martha Karen Newell

MARTHA KAREN NEWELL, Ph.D